Chromatographic Modeling of the Release of Particle-Adsorbed Molecules into Synthetic Alveolar Surfactant

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Pseudophase liquid chromatography was used to measure the thermodynamic parameters governing adsorption of organic molecules from the surfaces of carbonaceous particles into liposomal zwitterionic mobile phases. These mobile phases contain many of the important physicochemical parameters of alveolar surfactant. Results show that physical desorption into model surfactant will be dependent upon the heat of solution and the heat of adsorption. Dominance of either thermodynamic parameter is dependent upon the relative polarity of the adsorbent surface and the adsorbate molecule. It is postulated from data obtained from simple molecules containing relevant organic functional groups that physical desorption of environmental agents from the surfaces of particulate complexes into alveolar surfactant may be predicted both by quantification of the polarity of the system and of the extent of surface coverage under investigation.

Introduction

Respiration and alveolar deposition of carbonaceous particle-environmental agent complexes present the scenario for desorption of the surface-adsorbed molecules into regions of the lung not normally exposed to organic toxicants. The change in free energy when a particle-bound adsorbate is released into the surfactant-rich fluid found in the alveolar region of the lung will determine equilibrium concentrations of the adsorbate in the adsorbed and solution phases. If it is assumed that entropy contributions are small, enthalpy and bioavailability may be related in a dose-dependent manner. Chromatography is a useful tool to model the dynamic equilibrium of desorption of complexes typically formed by combustion processes.

This paper is the third in a series that describes research efforts to build a thermodynamic model to predict the bioavailability of adsorbed molecules on the surface of respirable particles. Prior research from this laboratory has investigated the release by high-performance liquid chromatography (HPLC), in which the adsorbent is the solid support, the adsorbate the solute, and the mobile phase pure solvents of a range of polarities (1). We have also quantified the enthalpies of the gas-phase adsorption of these same solutes onto the same carbon black adsorbents, which vary in degree of

oxidation and surface area (2). In a related investigation, we have successfully employed opaque micellar mobile phases in HPLC with pellicular C18 reverse-phase column packing materials (3). The results of these interrelated studies have allowed us to fully characterize the surface properties of these heterogeneous carbon black adsorbents and showed that carbon blacks behave as normal-phase chromatographic columns with nonpolar mobile phases but act as reverse-phase packings when polar mobile phases are used.

The synthetic alveolar surfactant used as a mobile phase in this investigation contains many of the relevant physicochemical properties of actual alveolar surfactant, since it is composed primarily of the zwitterionic phospholipid, dipalmitoyl phosphatidylcholine, which forms complex bilayer liposomes in aqueous solution. The chemical nature of the liposomal synthethic lung fluid indicates that it is likely to contain both polar and nonpolar regions and, therefore, desorption is postulated to be dependent upon the region of the liposome that contacts the particle-adsorbate complex. The primary physiological function of alveolar surfactant is to maintain the surface tension of the alveolus. Surfactant may be viewed conceptually as an epithelial coating, expanding and contracting with each respiration, washing over respired particles that become embedded in the alveolar epithelium.

Molecules that desorb into the surfactant may, therefore, have secondary contact with resident particles so that a toxic molecule may remain resident in

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the lung for a longer time than it would if it were diluted into alveolar surfactant and cleared via normal metabolic processes. The metabolic and cellular components of lung defense and clearance are not addressed in this study, and desorption is assumed to occur solely through physical processes. The mobile phases used in this study model human alveolar surfactant. Synthetic surfactant was used because it is impossible to obtain sufficient quantities of actual surfactant from animals by lung lavage. HPLC with liposomal mobile phases permits physical release to be modeled dynamically, although the mobile phase does present chromatographic difficulties that must be overcome in order to use ultraviolet detection. The method we have employed ensured that the carbon black adsorbents remained unaltered by the passage of these viscous mobile phases. Simple molecules that contain many of the important functional groups of biologically active molecules were used in our studies in order to model the mechanism involved in physical liposomal desorption. The role of the active site on the carbonaceous surface could thus be probed selectively.

The use of micellar mobile phases based on the surfactant sodium dodecyl sulfate for liquid chromatography (pseudophase chromatography) was first described by Armstrong (4). This approach to elution chromatography bridges the gap between partition and displacement chromatography. The solute may distribute between an aqueous solution of sodium dodecyl sulfate (the concentration of which corresponds to less than the critical micelle concentration), sodium dodecyl sulfate micelles, and the column packing material. Aqueous sodium dodecyl sulfate micelles are considered to have well-defined structures with negatively charged polar head groups at the water-micelle-interface and a nonpolar core. These micelles attract the sodium ions to their surfaces for electrical neutrality. The use of the resulting ionic complex for liquid chromatography can be considered to be analogous to ion chromatography. It is reasonable to expect that the resulting ion-pair will not interact directly with the column support because the surfaces of reverse-phase chromatographic packings are hydrophobic.

Two types of mobile phases, zwitterionic micelles and amphipathic liposomes, were used in this current study. Zwitterionic micelles were prepared from molecules that contain the basic quaternary ammonium ion and the acidic sulfonate ion of equal strengths. The aqueous solubilities (CMCs) of these Zwittergents are the result of the chain lengths of the alkyl group substituted on the quaternary ammonium ion. The liposomal mobile phase contained the major component of the surfactant found in the alveolar region of the lung, dipalmitoyl phosphatidylcholine, with minor amounts of dipalmitoyl phosphatidylethanolamine, cholesterol, and protein.

Both zwitterionic micelles and liposomes have zwitterionic polar head groups on their surfaces. The physical difference between these two systems is that the liposomes have aqueous cores and a bilayer structure with nonpolar interiors in the bilayers, whereas micelles have nonpolar cores and no bilayer structures. The concentrations of soluble zwitterionic species will vary since the CMCs of these systems are different and may, therefore, significantly affect the resulting chromatography.

Other researchers have used solution fluorescence spectroscopy to investigate the kinetics of the release of polynuclear aromatic hydrocarbons (particularly benzo[a]pyrene) from the surfaces of various carbon blacks into model phospholipid vesicles and rat lung homogenate (5–8). Phospholipid vesicles composed mainly of dimyristoyl or dipalmitoyl phosphatidylcholines were used as models for alveolar surfactant. These studies suggested that the surface area (coverage) may play a major role in the release of adsorbed molecules, since release increased with decreasing surface area. It was determined that only a portion of the adsorbed molecules could be released, and it is reasonable to propose, therefore, that the molecules which were released were less strongly bound (1,2). These studies quantified release kinetically by incubating the particle complex with the physiological or model solvents at physiological temperatures but made no attempt to quantify adsorption or desorption on the basis of thermodynamics.

Theory

A solute molecule that is introduced into a liposomic or micellar mobile phase that is passing over a column packing material will distribute in several ways. If the liposomic or micellar mobile phase interacts reversibly with the surface of the column packing material without producing concentration gradients of liposomes or micelles at the surface (i.e., liquid-solid chromatography is operating), then the following equilibria exist:
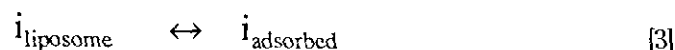
a) Distribution of the solute between aqueous phase and the liposomes or micelles

$$i_{\text{aqueous phase}} \quad \leftrightarrow \quad i_{\text{liposome}} \qquad [1]$$
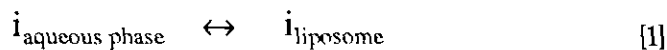
$$K^i_{\text{L,AQ}} = [i]_L/[i]_{AQ} \qquad [2]$$

The position of this equilibrium is based upon the lipophilicity of the solute. It will be independent of the column packing material but dependent upon the concentration of liposomes or micelles.

b) Distribution of the solute between the liposomes or micelles and the column packing material

$$i_{\text{liposome}} \quad \leftrightarrow \quad i_{\text{adsorbed}} \qquad [3]$$

$$K^i_{\text{A,L}} = [i]_A/[i]_L \qquad [4]$$

If the solute distributes preferentially into the liposomes or micelles, then any retention of the solute by the column packing material must be the result of the distribution of the solute between the liposomes or micelles and the column packing material. The contribution of this distribution will be dependent upon the liposome or micelle concentration and the properties of the column packing material.

c) Distribution of the solute between the aqueous phase and the column packing material

$$i_{aqueous\ phase} \leftrightarrow i_{adsorbed} \qquad [5]$$

$$K^i_{A,AQ} = [i]_A/[i]_{AQ} \qquad [6]$$

A hydrophilic solute (i.e., one that is present in the aqueous component of the mobile phase) may interact with the column packing material. The contribution of this interaction may be affected by changing the concentration of the liposomes or micelles in the mobile phase or by the type of column packing material.

Therefore, the overall distribution for the solute can be summarized by the following equilibrium:

$$i_{mobile\ phase} \leftrightarrow i_{adsorbed} \qquad [7]$$

$$K_{LSC} = [i]_A/[i]_{MP}$$

$$= \text{Function of } K^i_{L,AQ}, K^i_{A,L}, K^i_{A,AQ} \qquad [8]$$

If the liposomic or micellar mobile phase interacts irreversibly with the surface of the column packing material producing concentration gradients of liposomes or micelles at the surface (i.e., liquid-liquid chromatography is operating), then the following additional equilibria exist:

a) Distribution of the solute in mobile phase liposomes or micelles, and the thin film of liposomes or micelles, on the column packing material:

$$i_{liposome} \leftrightarrow i_{thin\ film} \qquad [9]$$

$$K^i_{F,L} = [L]_F/[L]_L \qquad [10]$$

If this distribution is a major contributor to the retention of the solute by liquid chromatography, then the solute must be interacting with the sorbed liposomes or micelles. Under these conditions, solutes will be retained as a function of their lipophilicities and as a function of the distribution of the liposomes or micelles between the mobile phase and the surface of the column packing materials. Liquid-liquid chromatog-

raphy must be responsible for the separation. Retention of lipophilic materials will increase with liposome or micelle concentration. Separation would tend to be independent of the column packing material once sufficient concentrations of liposomes or micelles are added to the mobile phase.

b) Distribution of the solute in the aqueous phase and the thin film of liposomes or micelles on the column packing material:

$$i_{laqueous\ phase} \leftrightarrow i_{thin\ film} \qquad [11]$$

$$K^i_{F,AQ} = [L]_F/[L]_{AQ} \qquad [12]$$

If this distribution is a major contributor to the retention of the solute by liquid chromatography, then the solute must be interacting with the sorbed liposomes or micelles. Under these conditions, solutes will be retained as a function of their lipophilicities and as a function of their distributions with the surface of the column packing materials. Liquid-liquid chromatography must be responsible for the separation. Retention of lipophilic materials may increase with liposome or micelle concentration. Separation would tend to be independent of the column packing material once sufficient concentrations of liposomes or micelles are added to the mobile phase.

Therefore, the overall distribution for the solute may be summarized by the following equilibrium:

$$i_{mobile\ phase} \leftrightarrow i_{thin\ film} \qquad [13]$$

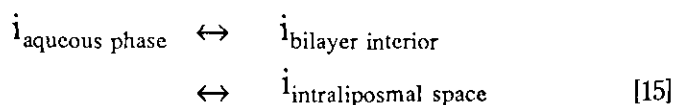$$K_{LLC} = [i]_F/[i]_{MP}$$

$$= \text{Function of } K^i_{F,L}, K^i_{L,AQ}, K^i_{F,AQ} \qquad [14]$$

These distributions may be summarized qualitatively. A lipophilic solute will distribute into the liposomes or micelles. Retention will decrease as the concentration of liposomes or micelles increases, providing that the liposomes or micelles do not sorb onto the surface of the column packing material. If the liposomes or micelles do sorb onto the surface of the column packing material, retention of lipophilic solutes will increase with increasing liposome or micelle concentrations.

The zwitterionic micelles used as mobile phases are quite different from the liposomal surfactant. A molecule that partitions across the polar head groups of the liposome into the nonpolar interior of the bilayer of a liposome will have an equal probability for repartitioning either across the polar head groups into the nonpolar interior of the liposome, or across the polar head groups into the extra-liposomal matrix. Therefore, a molecule will have an equal probability for

leaving the liposome or being carried with the liposome as it is transported by the stream of mobile phase. A micelle, by definition, has only one layer of polar head groups. A molecule that partitions into the micelle will either remain in the interior of the micelle, by virtue of its solubility in the nonpolar region, or it may partition back into the mobile phase. Therefore, liposomes include the following equilibria, which are, by definition, not present with micelles:

Distribution of the solute between the bilayer and aqueous phase, or between the bilayer and the intraliposomal space. The latter partitioning would require a solute molecule to cross the entire bilayer:

$$\overset{.}{i}_{aqueous\ phase} \quad \leftrightarrow \quad \overset{.}{i}_{bilayer\ interior}$$

$$\leftrightarrow \quad \overset{.}{i}_{intraliposmal\ space} \qquad [15]$$

$$K^{i}_{B,AQ} = [i]_{B}/[i]_{AQ} \quad or \quad K^{i}_{IS,B} = [i]_{IS}/[i]_{B} \qquad [16]$$

It has been established that every dipalmitoyl phosphatidylcholine molecule is hydrated with 23 molecules of water, 11 of which are in the interior, the remainder being associated with the head groups. The interior water molecules are so tightly bound that they do not freeze even at subzero temperatures. This suggests that the movement of solute molecules into the interior of the liposome will be sterically hindered (9). Therefore, the intercalation of nonpolar solutes into the phospholipid bilayer is the most probable transport mechanism. Proteins are also present in liposomal surfactant, acting as either integrated or associated proteins with the bilayer. Integrated proteins may facilitate partitioning of some molecules into the nonpolar interior of the liposome, especially if they extend completely across the bilayer. Associated proteins may themselves interact with the adsorbent surface, thereby altering the chromatography.

Experimental

Materials

Liposomal Surfactant Mobile Phases. Three different liposomes were used in this study, all prepared by identical procedures using commerically available compounds (Sigma Chemical Company): a) L-α-dipalmitoyl phosphatidylcholine (80.0 mg/mL); L-α-dipalmitoyl phosphatidylethanolamine (0.5 mg/mL); cholesterol (10.0 mg/mL); albumin (dog) (1.0 mg/mL); b) L-α-dipalmitoyl phosphatidylcholine (80.0 mg/mL; albumin (dog)(1.0 mg/mL); and c) L-α-dipalmitoyl phosphatidylcholine (80.0 mg/mL); L-α-dipalmitoyl phosphatidylethanolamine (0.5 mg/mL); albumin (dog) (1.0 mg/mL); cholesteryl oleate (10.0 mg/mL).

The constituents of each mobile phase, with the exception of albumin, were dispersed in a mixture of chloroform:methanol (2:1) (v:v). The solutions were evaporated to dryness under nitrogen to remove all organic solvents, leaving a uniform film on the wall of the vessel to be used in subsequent sonications. Tris buffer (0.01 M, pH 8.5) and the albumin were added, and the mixture was sonicated at 23°C for 30 min. This approach is based on the method proposed by Huang (9) and produces single-compartment liposomes. This stock solution of liposomes was then stored at 0°C until subsequent use. Aliquots of this concentrate were diluted with Tris buffer and gently vortexed.

Zwitterionic Micellar Mobile Phases. Two different zwitterionic surfactants were used in this study (Calbiochem Ltd.): Zwittergent 3-14 and Zwittergent 3-16. Their critical micelle concentrations (CMC) are 0.012% and 0.0012%, respectively. Standard dilutions of the Zwittergent surfactants in distilled deionized water were prepared, producing the following final concentrations: Zwittergent 3-14: 1.2%, 0.12%, and 0.012% (w/v); Zwittergent 3-16: 1.2%, 0.12%, 0.012%, and 0.0012% (w/v).

Adsorbates. The following adsorbates (Aldrich Chemical Company) were prepared as saturated decanted solutions in distilled deionized water and used in the experiments with liposomal surfactant: thiophene, benzaldehyde, acetophenone, nitrobenzene, benzofuran, quinoline, phenol, pyridine, p-benzoquinone, and aniline. For the experiments with zwitterionic micellar mobile phases, the following adsorbates (Aldrich Chemical Co.) were prepared as 0.01% (v/v) solutions in methanol: pyridine, benzaldehyde, nitrobenzene, benzofuran, and thiophene.

Adsorbents. The ASTM classifications of the carbon black adsorbents studied are as follows: N765, N339, and N339 oxidized (Cabot Corporation). Additionally, pellicular C18 and CN columns (5 cm × 4 mm ID) were used (Supelco, Inc.).

Temperature. For the experiments with liposomal surfactants, isothermal column temperature was maintained with an oil bath kept at constant temperatures of 22°C, 37°C, or 47°C. Only one temperature, 35°C, was used for the experiments with zwitterionic micelles.

Procedure

Synthetic Alveolar Liposomal Surfactant Experiments. Two liquid chromatographic pumps with a variable wavelength ultraviolet detector, λ_{254} for all compounds except thiophene, λ_{226} (Varian 2000 Series) were used to investigate adsorption-desorption phenomena of all adsorbates. Column packing techniques and column specifications were as previously described (1). Short columns (5 cm, 6 mm OD, 2 mm ID) were packed with the blacks (0.2 mm average particle size). Low flow rates (0.4 mL/min) were found to be stable for extended use.

Known aliquots (10 μL) of solutions of the adsorbates in water were introduced via a liquid sampling valve (Rheodyne 7125) onto the chromatographic column. The columns were conditioned with mobile phase prior to use. Adsorption was studied isothermally at different temperatures. The void volume of the column was determined by injection without a column, followed by injection with the column empty. This volume minus the volume occupied by the known weight of carbon contained in the column (based on the density of the carbon in the mobile phase) was used to calculate the void volume (V_{m}). The liquid chromatographic data were recorded on a chart recorder (Varian A 25).

Postcolumn clarification of the eluting sample-mobile phase was achieved by means of a stream (3.1 mL/min) of clarifying solvent (dichloromethane: methanol: acetonitrile, 1:1:1 v/v/v), via a second pump, connected to a specially designed low-volume connecting "Y." The eluting mixture of liposomal mobile phase and clarifying solvent then passed into a mixer consisting of a column (10 cm long, 0.5 mm ID) packed by gravitation with silanized glass beads (1000–1050 μm). Since clarification was produced postcolumn, no flow corrections were required.

Zwitterionic Micelles. A liquid chromatographic pump with a variable wavelength UV detector (λ_{254} for all compounds except thiophene, λ_{226}) (Varian 2000 Series), was used in the normal manner for these separations. Known aliquots (10 μL) of solutions of adsorbates were injected via a liquid sampling valve (Rheodyne 7125) onto the chromatographic column. The columns were conditioned with each mobile phase prior to use. The void volume of the column was determined by injection of an unretained transition metal salt. The liquid chromatographic data were recorded on a chart recorder (Varian A25).

Physical Characteristics of Carbon Blacks

The solvent densities of the blacks were determined by weighing the quantities of mobile phases required to fill a volumetric flask with and without known masses of carbon particles. Low vacuum was used to remove air entrained in the pores of the carbon particles. These densities were used for the determination of column void volumes. The other physical properties of these blacks have been reported previously (1,2).

Results and Discussion

The carbon black columns, as compared to the C18 and CN reverse-phase columns, required longer times to equilibrate with all the mobile phases before stable baselines were achieved. This indicates that the liposomal and micellar zwitterionic mobile phases are interacting more extensively with carbon surfaces, possibly with the free electrons present in amorphous

carbons. The retention data obtained at 37°C with C18 and CN packings using liposomes prepared from dipalmitoyl phosphatidylcholine, dipalmitoyl phosphatidylethanolamine, cholesterol, and albumin, are contained in Tables 1 and 2. Most of the solutes studied did not interact significantly with the CN packing material. The basic solutes (aniline, pyridine, and quinoline) did not interact with either the CN or the C18 columns, and retention did not change markedly when the concentration of liposomes in the mobile phase was increased. However, when the solute molecules are nonpolar (e.g., thiophene, nitrobenzene, and benzofuran), increasing liposomal mobile-phase concentration did produce changes in retention volumes. The variation in the capacity factors as a function of liposome concentration show maxima with the CN packing and a continued decrease for the C18 packing (Figs. 1 and 2).

These results suggest that there is some sorption of the liposomes onto the CN packing, whereas there is probably no significant sorption of the liposomes on the C18 packing. An interpretation of the maxima in the CN data could be that at low concentrations of liposomes, liquid-liquid chromatography is operating (i.e., the sorbed liposomes are acting as a thin film stationary phase, Eq. 14), and as concentration increases above 4%, the surface of the packing becomes saturated. No additional liposomes can coat the surface. Therefore, retention decreases as the concentrations (polarities) of the mobile phase and thin film stationary phases approach one another. The retention data on the C18 column may be explained by typical reverse-phase

Table 1. Capacity factors on CN column at 37°C

Solute	Concentration of liposomes			
	0%	4%	8%	12%
Thiophene	0.13	0.53	0.42	0.37
Benzaldehyde	0.13	0.10	0.10	0.10
Acetophenone	0.13	0.27	0.21	0.16
Nitrobenzene	0.13	0.74	0.42	0.42
Phenol	0.13	0.05	0.05	0.05
Benzofuran	0.54	2.27	1.27	0.90
Quinoline	0.27	0.27	0.21	0.21
Benzoquinone	0.13	0.05	0.05	0.05
Pyridine	0.13	0.05	0.05	0.05
Aniline	0.00	0.05	0.05	0.05

Table 2. Capacity factors on C18 column at 37°C.

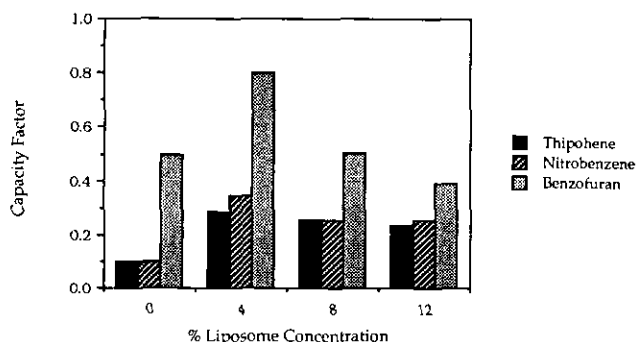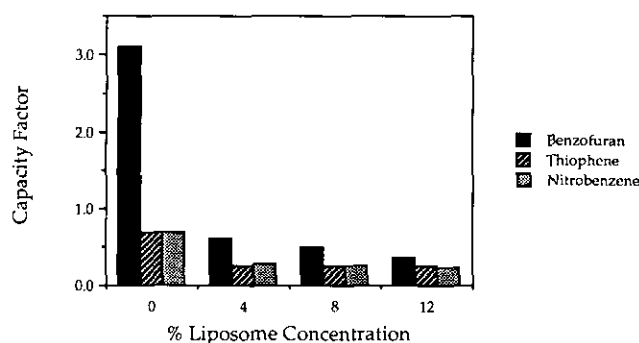| Solute	Concentration of liposomes			
	0%	4%	8%	12%
Thiophene	0.67	0.42	0.42	0.42
Benzaldehyde	0.40	0.16	0.10	0.21
Acetophenone	0.40	0.21	0.27	0.21
Nitrobenzene	0.67	0.53	0.47	0.37
Phenol	0.27	0.27	0.16	0.10
Benzofuran	3.04	1.63	1.27	0.84
Quinoline	0.80	0.37	0.37	0.21
Benzoquinone	0.13	0.05	0.05	0.05
Pyridine	0.00	0.05	0.05	0.05
Aniline	0.13	0.10	0.10	0.10

FIGURE 1. Retention data on Cn column with liposomes.

FIGURE 2. Retention data on C18 with liposomes.

chromatography (Eq. 8), since retention decreases as the polarity of the mobile phase approaches that of the C18 packing, although this decrease is approximately exponential and not linear.

Comparable retention data were obtained for the various carbon columns using the same liposomes. These data (Table 3) show similarities with the C18 packings, since solutes interacted less with the carbon surfaces as the liposome concentration was increased. There were a few exceptions to these general observations, such as phenol on N339 and pyridine and aniline on N110, for which minor maxima were observed. The addition of the liposomes significantly reduced the interactions with the surfaces of the carbon blacks, as demonstrated by the fact most solutes were eluted from these carbon surfaces. There were, however, some exceptions to this rule, since some nonpolar solutes were retained even at 12% liposome concentration. Comparison of these data to data previously obtained (2) with pure organic solvent mobile phases (1-hexane, dichloromethane, tetrahydrofuran, and methanol) shows that the surface active sites that did interact with basic solutes in pure organic mobile phases were completely deactivated by the liposomal phases. The elution properties of the solutes with liposomal mobile phases were comparable to those obtained when methanol or tetrahydrofuran were used as mobile phases.

The chromatographic retention of each of the individual components of the liposomes was studied using

Table 3. Equilibrium constants at 37°C for solutes as a function of carbon.

Solute	Concentration of liposomes			
	0%	4%	8%	12%
N765				
Thiophene	3.95	1.64	1.37	0.07
Benzaldehyde	B[a]	2.69	0.59	0.00
Acetophenone	R[b]	2.17	1.37	0.30
Nitrobenzene	B	3.22	0.59	0.50
Phenol	15.30	0.59	0.59	0.07
Benzofuran	11.50	2.95	0.85	0.07
Quinoline	R	3.47	0.85	0.00
Benzoquinone	52.87	0.07	0.00	0.00
Pyridine	2.76	0.07	0.07	0.00
Aniline	2.93	0.07	0.00	0.00
N339 ox				
Thiophene	R	2.69	0.59	0.32
Benzaldehyde	R	R	0.00	0.00
Acetophenone	R	B	0.00	0.00
Nitrobenzene	R	R	0.00	0.07
Phenol	R	1.37	0.85	0.00
Benzofuran	R	R	0.00	0.07
Quinoline	R	R	0.59	0.00
Benzoquinone	R	0.00	0.00	0.00
Pyridine	R	1.12	0.32	0.00
Aniline	R	1.12	0.07	0.00
N339				
Thiophene	R	6.30	3.22	1.90
Benzaldehyde	R	B	0.00	0.00
Acetophenone	R	B	B	B
Nitrobenzene	R	R	R	R
Phenol	R	2.95 (B)	2.95	4.52(B)
Benzofuran	R	R	R	R
Quinoline	R	R	R	R
Benzoquinone	R	2.42(B)	0.00	0.00
Pyridine	39.90	1.12	1.37	0.59
Aniline	R	2.95	1.37	1.37
N110				
Thiophene	R	R	R	3.74
Benzaldehyde	R	R	0.05	0.85
Acetophenone	R	R	R	R
Nitrobenzene	R	R	R	R
Phenol	R	R	B	B
Benzofuran	R	R	R	R
Quinoline	R	R	R	R
Benzoquinone	R	R	B	0.58
Pyridine	R	1.12	2.69	0.85
Aniline	R	1.12	3.74	1.64

[a]B, broad peak.

[b]R, retained irreversibly.

water as the mobile phase because there appeared to be minor sorption of the liposomes onto the surface of the carbon blacks. Dipalmitoyl phosphatidylethanolamine and cholesterol were found to be retained by the carbon columns, while the other components (dipal mitoyl phosphatidylcholine and albumin) were found to elute at the void volumes of the carbon columns.

Therefore, liposomes were prepared using only dipalmitoyl phosphatidylcholine and albumin to

Table 4. Equilibrium constants at 37°C for solutes as a function of carbon.

Solute	Concentration of liposomes (DPPC and albumin)						
	0%	0.5%	1%	2%	4%	8%	12%
N765							
Thiophene	3.95	2.17	1.90	1.64	1.39	1.12	1.90
Benzaldehyde	B[a]	7.15	4.00	1.64	1.12	0.32	0.59
Acetophenone	R[b]	10.6	6.37	1.52	1.12	0.32	0.82
Nitrobenzene	B	R	B	4.52	2.69	1.64	2.17
Phenol	15.30	1.90	1.37	0.85	0.59	0.07	0.32
Benzofuran	11.5	R	B	B	0.82	1.64	0.82
Quinoline	R	R	B	1.12	0.82	1.12	0.37
Benzoquinone	52.87	2.17	1.36	0.32	0.82	0	0.59
Pyridine	2.76	1.12	0.07	0.32	0	0.07	0.59
Aniline	2.93	1.37	0.59	1.12	1.37	0.07	0.07
N339							
Thiophene	R				B	B	R
Benzaldehyde	R				0.07	0.00	0.00
Acetophenone	R				B	B	0.00
Nitrobenzene	R				B	R	R
Phenol	R				5.05	1.37	0.00
Benzofuran	R				R	R	0.32
Quinoline	R				R	R	0.00
Benzoquinone	R				B	0.00	0.00
Pyridine	39.90				1.64	0.32	0.00
Aniline	R				3.22	1.12	0.00
N339 ox							
Thiophene	R	6.62	4.79	4.27	2.95	1.90	2.69
Benzaldehyde	R	0.85	0.85	B	B	0.07	0.07
Acetophenone	R	5.15	B	R	2.69	0.59	0.07
Nitrobenzene	R	R	R	R	R	R	R
Phenol	R	7.94	4.27	4.00	2.42	1.90	1.37
Benzofurane	R	R	R	R	R	R	1.12
Quinoline	R	R	R	R	R	R	B
Benzoquinone	R	R	B	8.47	3.74	2.69	0.00
Pyridine	R	2.95	2.69	2.17	1.64	1.37	0.00
Aniline	R	4.52	4.00	3.47	2.17	2.17	0.00

[a]B, broad peak.
[b]R, retained irreversibly.

investigate the mechanism responsible for the separation (referred to in tables and figures as "liposomes DPPC and albumin"). The results obtained with this liposomal mobile phase for three of the carbon blacks at additional liposome concentrations are contained in Table 4. Figure 3 shows the variation in distribution constants for selected solutes as a function of mobile phase concentration on N765. Comparison of these results to those given in Table 3 shows that the mobile phase which did not contain cholesterol or dipalmitoyl phosphatidylethanolamine did not moderate the solute interactions with the carbon surfaces as significantly. This suggests that there was less coating of the adsorbent by the mobile phase containing only dipalmitoyl phosphatidylcholine and albumin than by the mobile phase which contained all four components. Additional experiments were performed in which an aqueous mobile phase was moderated by the addition of albumin. No effect upon retention data were observed, suggesting that the albumin was

possibly acting merely as an associated protein with the liposome bilayer and did not, therefore, contribute to retention.

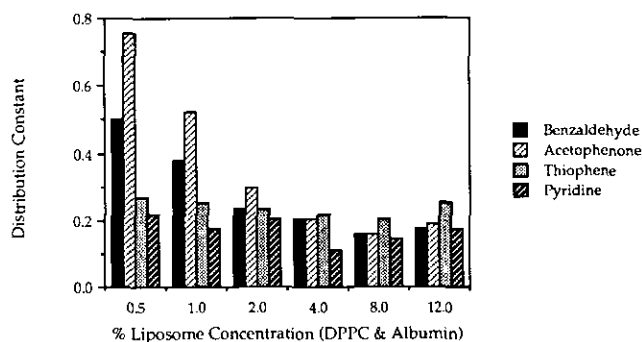In a subsequent series of experiments, cholesterol was

FIGURE 3. Distribution constants on N765 with liposomes (DPPC and albumin).

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Table 5. Equilibrium constants at 37°C for solutes as a function of carbon.

	Concentration of liposomes (ester)					
Solute	0%	0.5%	1%	2%	4%	8%
N765						
Thiophene	3.95	2.69	2.95	2.17	2.17	3.39
Benzaldehyde	B[a]	11.9	3.22	0.85	2.17	2.42
Acetophenone	R[b]	13.2	4.52	4.27	2.17	3.22
Nitrobenzene	B	B	6.37	7.42	5.32	4.27
Phenol	15.30	1.64	1.37	1.90	2.17	1.37
Benzofuran	11.5	B	B	14.5	9.25	B
Quinoline	R	13.2	6.62	5.84	4.52	4.00
Benzoquinone	52.87	0.59	1.12	0.32	0.32	0.59
Pyridine	2.76	0.59	1.12	1.12	0.59	0.82
Aniline	2.76	0.85	1.64	1.37	1.12	1.90
N339 ox						
Thiophene	R	4.79	4.79	3.22	2.69	B
Benzaldehyde	R	46.0	33.2	17.9	14.0	7.15
Acetophenone	R	R	R	B	12.9	B
Nitrobenzene	R	R	R	R	R	B
Phenol	R	6.89	6.10	5.84	3.22	2.17
Benzofuran	R	R	R	R	R	B
Quinoline	R	R	R	R	R	B
Benzoquinone	R	R	11.1	6.62	4.79	2.69
Pyridine	R	4.00	2.95	2.42	1.64	1.64
Aniline	R	5.57	4.52	4.00	2.42	1.64

[a]B, broad peak
[b]R, retained irreversibly.

replaced by cholesteryl oleate, because the cholesterol in alveolar surfactant is probably esterified. Cholesteryl oleate is less polar than the free sterol and, therefore, should interact more weakly with carbon surfaces. Liposomes were prepared from cholesteryl oleate, dipalmitoyl phosphatidylcholine, dipalmitoyl phosphatidylethanolamine, and albumin [referred to in tables and figures as "liposomes (ester)"]. The results obtained by the addition of these liposomes to water for two of the carbon adsorbents are listed in Table 5. Liposomes (ester) appear to moderate the aqueous mobile phases less than the other two compositions of liposomes (Tables 3 and 4), which supports the hypothesis of reduced interaction of the cholesterol ester with the carbon black adsorbents. This effect is illustrated by the data presented in Figure 4.

Retention data for these three liposomal mobile phases were determined at additional temperatures in order to obtain values for the heats of adsorption from
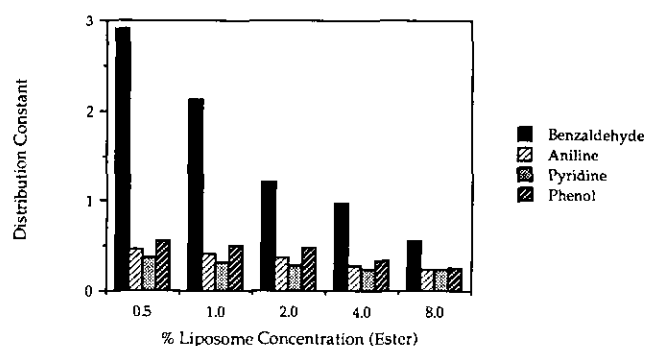

FIGURE 4. Distribution constants on N339 ox with liposomes (ester).

Tables 6 through 10. Initially, 22°C and 37°C were used to obtain these values; however, it was found that the heats of adsorption were much higher when ambient temperature and 37°C were used than when 37°C and 47°C were used (Tables 6–9). Dipalmitoyl phosphatidylcholine has a quasi-fluidic phase transition around 33°C, and this transition probably contributes to the values for the heat of adsorption. In general, the heats of adsorption are positive. This suggests that the heats of solution of the solutes in the liposomes are significant contributors to the overall heat of adsorption obtained by liquid chromatography. The magnitude of the heat decreased with increasing liposome concentration. Comparison of the data in Tables 6 through 10 shows that the heats are lower for the liposome (ester) mobile phases. As the carbon surface is changed progressively from N765 (small numbers of active sites) to N110 (greatest number of active sites), there are more extensive interactions with the carbon surfaces by the solutes under investigation (1). These data demonstrate that there can be significant interaction between the adsorbate and the adsorbent surface for some of the carbon surfaces, even in the presence of mobile phases that can coat the surface of the adsorbent. Therefore, liquid-solid chromatography is the dominant process by which solutes interact.

Human alveolar surfactant contains additional constituents such as phosphatidylglycerol, phosphatidylinositol, sphingomyelin, phosphatidylserine, and immunological agents. The selection of phosphatidylethanolamine was predicated upon the fact that there appeared to be good agreement in the literature as to the percentage of this lipid in surfactant,

Table 6. Heat of adsorption as a function of liposome concentration for N765, N339, N339 ox, and N110.

Adsorbate	Mobile phase composition[a]											
	N765			N339			N339 ox			N110		
	4%	8%	12%	4%	8%	12%	4%	8%	12%	4%	8%	12%
Thiophene	40	35	29	B[b]	1.5	-5.8	0	27	6.8	R[c]	R	27
Benzaldehyde	83	53	40	R	-12	B	R	0	-22	R	29	33
Acetophenone	77	52	60	R	R	R	B	-19	-12	R	R	R
Nitrobenzene	73	63	27	R	R	R	R	R	R	R	R	R
Phenol	53	39	53	B	B	27	R	16	-3.7	R	B	B
Benzofuran	55	47	44	R	R	R	R	-25	-6.8	R	R	R
Quinoline	90	35	53	R	R	R	R	R	R	R	R	R
p-Benzoquinone	44	31	40	R	R	14	-77	-28	-3.7	R	R	R
Pyridine	53	79	31	-2.3	-6.8	-11	14	-8.7	-11	3.1	5.6	28
Aniline	63	67	40	-8.7	-12	0	23	-16	-3.7	12	27	45

[a]295-310°K.
[b]B, broad peak.
[c]R, retained irreversibly.

Table 7. Heat of adsorption as a function of liposome concentration for N765.

Adsorbate	Mobile phase composition											
	295-310°K						310-320°K					
	0.5%	1%	2%	4%	8%	12%	0.5%	1%	2%	4%	8%	12%
Thiophene	-5.5	42	32	43	48	40	-12	0	15	17	28	20
Benzaldehyde	-9	40	67	60	46	40	-12	0	15	17	28	20
Acetophenone	60	79	54	60	46	56	-11	-11	45	31	44	13
Nitrobenzene	R[a]	B[b]	67	59	55	53	R	B	9.2	19	32	4.5
Phenol	0	17	35	31	41	51	-5.4	5.8	18	26	16	39
Benzofuran	R	B	B	35	47	56	R	R	B	97	49	46
Quinoline	R	B	48	56	44	46	R	B	93	57	57	53
p-Benzoquinone	22	43	46	56	17	66	4.5	-5.8	28	-15	45	-16
Pyridine	8.6	21	35	28	41	51	-6.7	42	34	29	8.3	7.3
Aniline	7.7	23	60	63	41	41	-5.8	26	0	0	8.3	16

[a]R, retained irreversibly.
[b]B, broad peak.

Table 8. Heat of adsorption as a function of liposome concentration for N339 ox.

Adsorbate	Mobile phase composition											
	295-310°K						310-320°K					
	0.5%	1%	2%	4%	8%	12%	0.5%	1%	2%	4%	8%	12%
Thiophene	8	30	14	40	10	33	-2.3	-9.7	0	-4.3	-17	-53
Benzaldehyde	-7.7	-7.7	B[a]	B	0	6.1	184	B	B	B	81	-67
Acetophenone	R[b]	R	R	R	10	0	R	R	R	B	68	-21
Nitrobenzene	R	R	R	R	R	R	R	R	R	R	R	R
Phenol	25	2.1	21	30	24	28	-22	3.1	-11	4.5	-11	-57
Benzofuran	R	R	R	R	R	19	R	R	R	R	R	-40
Quinoline	R	R	R	R	R	B	R	R	R	R	R	B
p-Benzoquinone	R	B	39	42	51	-6.1	R	B	-31	0	-82	-24
Pyridine	0	16	22	21	12	0	4.1	-4.5	-16	-12	-36	-24
Aniline	4.0	15	23	14	18	6.9	12	3.4	-3.7	-5.1	-36	-24

[a]B, broad peak.
[b]R, retained irreversibly.

whereas there appears to be some disparity in the literature as to the percentage of phosphatidylglycerol. King reports that canine pulmonary surfactant contains 5% phosphatidyglycerols (11); Pfleger and Thomas (12) and Kikkawa and Smith (13) report 10%. The phosphitidylethanolamine content of canine surfactant ranges from reported values of 7.2 to 3% (11,13). Both phosphatidylglycerol and phosphatidyl-

ethanolamine are present in relatively low concentration in lung surfactant compared to the phosphatidylcholine content. Our studies were concerned with phosphatidylcholine, the primary lipid present in alveolar surfactant. Phosphatidylethanolamine was added only to demonstrate that the minor ingredients do indeed play a role in modifying release.

Studies were also performed using zwitterionic

Table 9. Heat of adsorption as a function of liposome concentration for N339.

Adsorbate	Mobile phase composition						
	295–310°K				310–320°K		
	4%	8%	12%		4%	8%	12
Thiophene	R[a]	R	R		R	R	R
Benzaldehyde	R	20	11		B[b]	B	B
Acetophenone	R	B	20		R	B	–9.1
Nitrobenzene	R	R	R		R	R	R
Phenol	R	35	8.8		8	11	–9.1
Benzofuran	R	R	35		R	R	–99
Quinoline	R	R	24		R	R	–116
p-Benzoquinone	R	8.8	11		B	0	B
Pyridine	–3.3	26	0		5.4	0	–146
Aniline	11	32	20		0	–6.7	–91

[a]R, broad peak.
[b]B, retained irreversibly.

Table 10. Heat of adsorption as a function of liposome concentration for N765 and N339 ox.

Adsorbate	Mobile phase composition[a]			
	N765		N339 ox	
	4%	8%	4%	8%
Thiophene	0	–17	R[b]	B[c]
Benzaldehyde	–36	–33	47	39
Acetophenone	0	–34	B	B
Nitrobenzene	–12	–32	R	R
Phenol	–22	–20	7.4	4.5
Benzofuran	–10	B	R	R
Quinoline	–6.5	–15	R	B
p-Benzoquinone	–8.3	–8.0	30	8.4
Pyridine	–16	–24	–5.8	–18
Aniline	–22	–39	–9.6	–26

[a]310–320°K.
[b]R, retained irreversibly.
[c]B, broad peak.

Table 11. Capacity factors on CN column at 35°C.

Solute	0.012%	0.12%	1.2%
Concentration of Zwittergent 3-14			
Thiophene	0.30	0.30	0.19
Benzaldehyde	0.26	0.15	0.11
Nitrobenzene	0.33	0.37	0.19
Benzofuran	2.00	1.70	0.30
Pyridine	0.19	0.11	0.07
Concentration of Zwittergent 3-16			
Thiophene	0.30	0.19	0.37
Benzaldehyde	0.22	0.15	0.22
Nitrobenzene	0.19	0.19	0.41
Benzofuran	2.41	2.33	1.67
Pyridine	0.11	0.11	0.19

micellar mobile phases (Zwittergent 3-14 and 3-16) at concentrations exceeding their critical micelle concentrations. It was hoped that these mobile phases would be chromatographically and physicochemically similar to the liposomes but would expedite experimentation since no postcolumn clarification would be required. The data obtained for these mobile phases are shown in Table 11, and it may be seen that there was indeed some similarity between these data and the data shown in Table 2. However, when attempts were made to use these mobile phases with the carbon columns, it was impossible to obtain stable detector signals. The carbon columns started to break down and became unstable with these mobile phases. This instability may be due to differences in the critical micelle concentrations of the Zwittergent (3-14 CMC = 0.012%, 3-16 CMC = 0.0012%) as compared to dipalmitoyl phosphatidylcholine (CMC = 1 × 10⁻¹⁰M). Therefore, experimental studies with these mobile phases were discontinued.

It may be postulated that most of the simple molecules used in this study will be desorbed from the surfaces of carbon blacks by actual alveolar lung surfactant (approximately 99% dipalmitoyl phosphatidylcholine

liposomes). The time for release will be dependent upon the strength of interaction of the molecule with the adsorbent surface and the heat of solution for the adsorbate molecule in alveolar lung surfactant. Therefore, it is possible that these molecules may be desorbed to produce either acute, short term (rapid release), or long-term chronic (slow release) doses of the agents to the lung tissue and cells.

Conclusions

These studies provide insight into the relative probability for the desorption of particle-adsorbed molecules into alveolar surfactant by correlating desorption with bioavailability. It is unlikely that the particle will act as a sink for polar molecules in solution in the alveolar surfactant once physical release of an adsorbed molecule from the particle surface has occurred. The layer of alveolar surfactant will coat the particle and block the sites on the carbon surface. However, the situation is the opposite for totally non-polar molecules in solution in the alveolar surfactant, which would tend to be sorbed onto the surfaces of *in situ* nonpolar particles. This event could enhance the residence time of nonpolar molecules in the lung. Phagocytic cells such as alveolar macrophages could provide the means for metabolic release of nonpolar

molecules. Postphagocytic events may lead to macrophage lysis and release of any unmetabolized nonpolar molecules onto the lung epithelium, which is the deposition and residence site of the carbonaceous particle prior to clearance.

A nonpolar molecule is known to be metabolized within phagocytic cells to a more polar metabolite, thus facilitating detoxification. If a cell containing such hydrophilic metabolites of nonpolar adsorbates is lysed, the cellular contents would not be predisposed to readsorption onto any carbonaceous particle surface. The metabolites would, therefore, be less likely to be re-ingested by phagocytes. Polar metabolites are likely to remain in solution in the alveolar surfactant, and would, therefore, have increased probability for interaction with lung epithelium. Nonpolar molecules that are metabolized more slowly could be cycling between alveolar surfactant and resident carbonaceous particles and may, therefore, remain in deep lung for longer periods of time. Residence time will be further enhanced if the carbonaceous particle does not elicit a significant inflammatory response leading to an increased influx of phagocytic cells to the lung.

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